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Abstract

A decade after environmental scientists integrated high-throughput sequencing technologies in their toolbox, the genomics-based monitoring of anthropogenic impacts on the biodiversity and functioning of ecosystems is yet to be implemented by regulatory frameworks. Despite the broadly acknowledged potential of environmental genomics to this end, technical limitations and conceptual issues still stand in the way of its broad application by end-users. In addition, the multiplicity of potential implementation strategies may contribute to a perception that the routine application of this methodology is premature or “in development”, hence restraining regulators from binding these tools into legal frameworks. Here, we review recent implementations of environmental genomics-based methods, applied to the biomonitoring of ecosystems. By taking a general overview, without narrowing our perspective to particular habitats or groups of organisms, this paper aims to compare, review and discuss the strengths and limitations of four general implementation strategies of environmental genomics for monitoring: (A) Taxonomy-based analyses focused on identification of known bioindicators or described taxa; (B) *De novo* bioindicator analyses; (C) Structural community metrics including inferred ecological networks; and (D) Functional community metrics (metagenomics or metatranscriptomics). We emphasise the utility of the three latter strategies to integrate meiofauna and microorganisms that are not traditionally utilised in biomonitoring because of difficult taxonomic identification. Finally, we propose a roadmap for the implementation of environmental genomics into routine monitoring programs that leverage recent analytical advancements, while pointing out current limitations and future research needs.

The need for broad scale ecosystem monitoring strategies

Biodiversity drives the fundamental processes of ecosystems and provides invaluable services on which we depend. Anthropogenic, detrimental impacts on ecosystems, including accelerating climate change, are unprecedented (Waters et al., 2016) and have led to a decline of biodiversity across the globe (Butchart et al., 2010; Cardinale et al., 2012; Hughes et al. 2018). Recent reports stress that one out of the 8 million known species are presently at risk of extinction (IPBES report, 2019). This threatens ecosystem function(ing) and services. Therefore, the urgent challenge is now to build a set of efficient tools to enhance our capacity to predict or detect early warnings of critical ecological shifts efficiently, in order to forecast the direction of such shifts and their impacts on ecosystem functions and services (Carpenter et al., 2011; Barnosky et al., 2012; Ratajczak et al., 2018).

Because our societies aim to reach a trade-off between socioeconomic development and ecosystems sustainability (UN A/RES/70/1, 2015), regulatory frameworks have been established worldwide for the sustainable development of industries within environmental constraints (Niemeijer 2002; Grizetti et al., 2015). Such regulatory systems have been incorporated into various national and international directives, especially for aquatic ecosystems (e.g. the Water Framework Directive, WFD, Directive 2000/60/EC and Marine Strategy Framework Directive, MSFD, Directive 2008/56/EC in Europe, the Clean Water Act of the US Environmental Protection Agency in the USA, as well as the United Nations Convention on the Law of the Sea, UNCLOS). The backbone of such monitoring programs is the biological component of ecosystems, as a measure of ecosystem ‘health’ or ‘integrity’ (Karr, 1999). This biological component is often referred to as the Biological Quality Elements in those regulations (BQEs, Borja et al., 2013; Hering et al., 2018). Most monitoring strategies implemented in regulations rely on the bioindication principle (autecology, Box 1), i.e. significant correlations between the occurrence of specific organisms and a set of environmental variables. Although chemical and hydrological monitoring techniques provide an environmental quality snapshot, biological indicators convey a cumulative time-integrated measure as their occurrence is the product of their local adaptation and their responses to ecosystem variations and/or disturbances across an extended period of time (Carignan & Villard, 2002; Lear et al., 2011; Birk et al., 2012).

The limits of currently implemented ecosystem monitoring strategies

Traditionally, morphologically distinguishable invertebrates have been used as bioindicators in both aquatic and terrestrial ecosystems (Reynoldson & Metcalfe-Smith, 1992; Bongers & Ferris, 1999; Hodkinson & Jackson, 2005; Gerlach et al., 2013). Fishes, amphibians, macrophytes, phytoplankton and diatoms, are also routinely used in aquatic ecosystems (Birk et al., 2012). Various Biotic Indices (BIs) have been formalized, based on the predictable responses of bioindicator species to environmental disturbances (autecological value) in marine (Maurer et al., 1999; Borja et al., 2000; Rygg et al., 2013), freshwater (Kelly et al., 1995; Stark et al., 1998; Prygiel & Coste, 2000) and terrestrial (Urzelai et al., 2000; Marull et al., 2007) ecosystems. Almost half of the monitoring methodologies currently used in Europe rely on such BIs (Birk et al., 2012). However, for environments or geographical regions for which no BI has been calibrated, ecological assessments rely instead on biodiversity measures of “charismatic” groups such as fishes (Pont et al., 2006), amphibians (Welsh et al., 1998) and insects (Basset el al., 2004).

Morphology-based methodologies require the collection and identification of hundreds to thousands of specimens per sample, which is a slow, labor-intensive process. These limitations seriously hamper our capacity to scale up biomonitoring and satisfy the increasing demand for environmental monitoring programs in a timely fashion that allows informed ecosystem management (Baird & Hajibabaei, 2012). Moreover, this conventional morphology-based approach is compromised by several other shortcomings: (i) it focuses only on morphologically identifiable biodiversity, ignoring the inconspicuous meiofaunal and microbial domains, which are known to include powerful bioindicators; (ii) cryptic diversity remains unrecognized (morphologically indistinguishable look-alikes with differing tolerance to disturbances); (iii) variation in species life stages, damaged specimens and misidentifications caused by decreasing taxonomic expertise worldwide may lead to variable and noisy species’ inventories, and by extension, to uncertain ecological assessments. Taken together, the need for faster, more objective, robust and cost-effective tools and strategies to deliver a more efficient ecosystem monitoring has never been more pressing.

The environmental genomics revolution for biodiversity research and ecosystem monitoring

Over the last decade, the development of environmental genomics (EG) coupled with high-throughput sequencing (HTS) technologies has led to a marked improvement in our ability to document biodiversity patterns, for both species occurrence (amplicon sequencing, i.e. metabarcoding, reviewed in Bohmann et al., 2014; Valentini et al., 2016; Deiner et al., 2017; Cristescu et al., 2018; Taberlet et al. 2018; Ruppert et al., 2019) and their metabolic functions (metagenomics and metatranscriptomics, reviewed in Ungerer et al., 2008; Vandenkoornhuyse et al., 2010; Quince et al., 2017; Singer et al., 2017; Escalas et al., 2019). Multidisciplinary teams and consortiums have initiated large scale projects aiming at collecting biodiversity data using EG throughout the globe, to address fundamental ecological questions. Among these initiatives, the large barcoding projects led by the international Barcode of Life (Ratnasingham & Hebert, 2007), the Earth Microbiome Project (Gilbert et al., 2010) and the TARA Oceans Project (Karsenti et al., 2011) represent three of the most emblematic examples. Those projects have unraveled an unexpected cryptic (Bickford et al., 2007) and novel microbial diversity (the 'unseen majority') guiding reconstruction of the eukaryotic tree of life (Adl et al., 2019). Even though this microbial diversity is known to represent a key component of ecosystem functioning (Delgado-Baquerizo et al., 2016; Guidi et al., 2016; Cavicchioli et al., 2019), the ecology of most microorganisms remains largely enigmatic.

The potential of EG for surveying biodiversity and monitoring natural ecosystems at a broad spatio-temporal scale was quickly identified and implemented by environmental scientists (Baird & Hajibabaei, 2012; Taberlet et al., 2012; Davies et al., 2012; Kelly et al., 2014). This work has been boosted by the massive drop in sequencing costs, with over four orders of magnitude within the last 15 years (https://www.genome.gov). This has enabled numerous clinical and environmental routine applications. Indeed, fueled by the continuous efforts to optimize laboratory protocols and bioinformatic tools, all steps from large-scale collection of samples, generation of HTS data, statistical analysis, and interpretation of results, can now be performed in matter of days or weeks (Juul et al., 2015; Quinn et al., 2016; Deshpande et al., 2019; Reintjes et al., 2019). For aquatic ecosystems especially, the next breakthrough of this revolution is now expected to be the development and deployment of low-cost, automated and miniaturized *in situ* environmental nucleic acids (eDNA/RNA) samplers (Carr et al., 2017; Gan et

al., 2017). These may be integrated into autonomous instruments for broad-scale and continuous ecosystem monitoring programs (Brandt et al., 2016; Bohan et al., 2017; Aguzzi et al., 2019; Benway et al., 2019; Levin et al., 2019).

These advances in genomics-based research have led to a series of pilot studies assessing the applicability of EG for the monitoring of ecosystem changes by collecting biodiversity data from various taxonomic groups (e.g. fishes, macroinvertebrates, protists, bacteria) and environments (e.g. water, biofilms, soil or sediment). Several such pilot studies have targeted multicellular organisms as a replacement for arduous morphological identification of the same taxa (Hajibabaei et al., 2011, 2012; Thomsen et al., 2012; Zhou et al., 2013; Lejzerowicz et al., 2015). However, the potential of EG to leverage the general eukaryotic and prokaryotic diversity for ecological monitoring, has also been explored (Chariton et al., 2010; Bik et al., 2012; Dowle et al., 2015; Lallias et al., 2015), and indeed advocated (Creer et al., 2010; Payne, 2013; Bouchez et al., 2016; Chariton et al., 2016; Graham et al., 2016). Encouraged by the immense opportunities for ecosystem monitoring, over 45 countries recently decided to join their efforts within the European COST Action DNAqua-Net, to anticipate upcoming paradigm-shifts and develop genomic tools tailored for the monitoring of aquatic ecosystems (http://dnaqua.net, Leese et al., 2016). Similarly, other large-scale collaborative projects were recently launched, including STREAM in Canada (https://stream-dna.com/), Lakes380 in New Zealand (https://lakes380.com/) and NGB in France (http://next-genbiomonitoring.org/), aiming at the unbridling of EG for ecosystem monitoring.

Multiple pilot and methodological EG studies have highlighted important variation in terms of compliance with current regulatory programs (reviewed in Hering et al., 2018), leading to the proposition of multiple implementation strategies for current and future ecosystem monitoring programs. Here, we compare and review the strengths and limitations of these EG-based strategies for ecosystem monitoring. Our objective is to pinpoint the criteria of existing monitoring programs that could be fulfilled by EG methods as of today, and clarify the work ahead for the monitoring programs that could benefit from EG in the near future, given continued technological and analytical advancements. To this end, we classify these strategies into four broad categories (Figure 1, Table S1): (A) Taxonomy-based analyses that focus on known bio-indicator species, or the identification and enumeration of formally or informally described taxa; (B) *De novo* bioindicator analyses aiming to identify and utilise novel

bioindicators, independent of formal taxonomy; (C) Structural community metrics relying on community structure or inferred ecological networks, where taxa are interchangeable; and (D) Functional community metrics or indicators that focus on protein-coding genes or transcripts instead of taxonomic composition. Based on the specificities of each strategy, their level of maturity and their compatibility with existing regulations (Table 1), we propose an implementation roadmap to integrate EG into ecosystems monitoring programs and highlight future research needs to be undertaken.

"Taxonomy-based" strategy: screening known species and bioindicators with environmental genomics

This strategy relies on the enumeration of known biodiversity from DNA obtained from an environmental sample (e.g. sediment, soil, biofilm, water) or from bulk material prepared from an environmental sample by e.g. elutriation, trapped individuals or biofilm scratching (Figure 1A). This strategy closely fits the conventional, morphology-based monitoring approach, because it primarily aims at reaching a satisfactory level of congruence in terms of both qualitative and quantitative biodiversity inventories. The taxonomy-based strategy is *de facto* limited to the morphologically characterized fraction of biodiversity for which reference sequences are available in public databases. Hence, approaches using it have usually overlooked meiofaunal or microbial taxa, difficult to identify on the basis of morphological traits, and for most of which the autecology is poorly known (but see Pawlowski et al., 2016). The reference databases routinely used by EG studies include for instance the universal but essentially non-curated GenBank nucleotide repository from the National Center for Biotechnology Information (Benson et al., 1999, but see Leray et al., 2019), or the curated databases BOLD for COI barcodes, primarily from animals (Ratnasingham & Hebert, 2007), SILVA for universal ribosomal markers (Quast et al., 2013), PR^2 for protists (Guillou et al., 2013), Diat.barcode for diatoms (Rimet et al., 2016), and Unite for fungi (Nilsson et al., 2018).

Depending on the environment assessed and the taxonomic group considered, the performance of taxonomy-based approaches varies considerably (Hering et al., 2018). Benchmarking studies comparing EG-based and conventional morphology-based taxonomic inventories (Table S1) have shown mixed degrees of congruence. For the non-invasive

detection of fish species from DNA traces in filtered marine water, the rate of success from taxonomy-based monitoring is reported near perfect (Thomsen et al., 2012; Bakker et al., 2017; but see DiBattista et al., 2017). For freshwater macroinvertebrate bulk samples, the rate of species detection varied from 67% (Elbrecht et al., 2017) to 73-83% (Hajibabaei et al., 2011; 2012). In contrast, for benthic diatoms sampled from biofilms, the congruence of morphological taxonomy and EG-inferred taxonomy, in terms of shared taxa at species level, ranged only from 15-18% (Rivera et al., 2017; Vasselon et al., 2017a) to 28% (Visco et al., 2015). The reported congruence for macroinvertebrates sampled from marine sediments ranged from 20% (Lejzerowicz et al., 2015) up to 60% (Aylagas et al., 2016). Noteworthy, those studies also detected numerous species that were unnoticed in morphological inventories (Hajibabaei et al., 2011; 2012; Elbrecht et al., 2017). Despite these discrepancies, the studies inferring BI values from the detected bioindicators species show very promising results, for both freshwater diatoms (Kermarrec et al., 2014; Visco et al., 2015; Vasselon et al., 2017b; Kelly et al., 2018) and macroinvertebrates (Elbrecht et al., 2017) as well as for marine macroinvertebrates (Lejzerowicz et al., 2015; Aylagas et al., 2016). While acknowledging that the congruence for both qualitative and quantitative inventories are not fully satisfactory, these studies have demonstrated that EG tools are still able to detect sufficient bioindicator taxa to infer accurate BI values, even when considering only presence/absence (Aylagas et al., 2016). The EG methodology has therefore been promoted as a promising tool for fast and cost-effective biodiversity screening for ecosystem monitoring, even while the simultaneous collection of classical morphological samples for validation is univocally suggested. Nonetheless, further improvements in molecular protocols as well as BI inter-calibration is a necessity towards harmonization and standardization across Europe (Poikane et al., 2014; Hering et al., 2018) and beyond (Jeunen et al., 2019).

Various biological and technical limitations still impede the implementation of the taxonomy-based strategy for routine monitoring applications (Leese et al., 2018). These limitations mainly stem from the fact that the methods sample fundamentally different units of presence (molecules *versus* individuals), resulting in different biases affecting richness, abundance and taxonomic composition. The richness of “molecular species”, i.e. Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs, the new operational unit paradigm, Callahan et al., 2017), should not be considered analogous to morpho-species

richness even in the theoretical absence of noise resulting from PCR and sequencing biases. This discrepancy is due to cryptic diversity (Stork, 2018), intragenomic or intra-specific marker variation (Bik et al., 2013, Sun et al., 2013), and the presence of DNA from dead and inactive organisms or as extracellular DNA (Collins et al., 2018). Likewise, the abundance of taxa inferred from HTS read counts can typically not be used to infer the number of individuals. Indeed, the number of sampled DNA molecules and sequence reads are a consequence of the number of individuals, but also of the biomass and the variable number of copies of the targeted marker in the genome (Bik et al., 2013, Vetrovský, et al., 2013), in addition to variations in DNA extractability and primer-specific amplification bias (Elbrecht et al., 2015; Piñol et al., 2015; Krehenwinkel et al., 2017). Finally, EG studies suffer from a strong sampling effect because DNA extractions are typically performed from small amounts of material, making large-size organisms less well-represented in eDNA extracts (Lanzén et al., 2017). However, bulk samples (Elbrecht et al., 2017), larger extraction volume (Nascimento et al., 2018) or more aggressive homogenization (Lanzén et al., unpublished data) can partially alleviate this issue.

Since the taxonomy-based strategy depends on reference sequences for organism identification, the incompleteness of reference databases can also have a major impact. Hence, completing databases, both by the “vertical” addition of more taxa and by the “horizontal” coverage of wider geographical areas, would certainly contribute to an improvement in identification (Vasselon et al., 2017; McGee et al., 2019). However, despite sustained efforts, reference databases will likely remain skewed towards some taxa, while suffering from important gaps across other taxonomic groups or biogeographical regions (Weigand et al., 2019; McGee et al., 2019). All these issues directly impact both of the key parameters for applying BIs to assess impact, namely the qualitative and quantitative measures of biodiversity (Pawlowski et al., 2018).

Nevertheless, multiple studies have shown that there is room for considerable improvements to better bridge the current gaps between taxonomy-dependent molecular and morphology-based approaches. Taxonomic breadth in HTS data could be broadened by carefully designing novel amplification primers (Elbrecht et al., 2019) or using more than one primer pair (Corse et al., 2019). Applying correction factors to read counts, based on established knowledge of the biovolume (Vasselon et al., 2018), the number of copies of the targeted marker (Vetrovský, et al., 2013) or by spiking samples with known internal standard for

quantitative determinations (Tkacz et al., 2018; Ji et al., 2019), are all promising methods for resolving these challenges. Finally, the integration of bioinformatic tools for the automated curation of databases from mislabeled sequences will improve their reliability (Ashelford et al., 2005; Kozlov et al., 2016).

"*De novo"* strategy: discovering new bioindicators and harnessing them for routine monitoring.

In contrast to the taxonomy-based strategy, the *de novo* one does not immediately generate an ecological assessment, because it does not employ previous knowledge associated with bioindicators. Instead, the *de novo* strategy aims at establishing new bioindicators using EG-based profiling of communities and independently generated ecological status or known disturbance gradients (Figure 1B). Harnessing EG and HTS technologies to explore a broader range of biological diversity, formally labelled or not (i.e. taxonomically described or identified), represents an opportunity to move towards a more holistic monitoring paradigm (Chariton et al., 2010; Bik et al., 2012). By considering all the OTU (or ASV) profiles along a known impact gradient of typical anthropogenic origin, studies applying this strategy have shown that HTS data represent a virtually unlimited reservoir of new bioindicators. Examples (listed in Table S1) include contamination by pesticides (Thompson et al., 2016; Andùjar et al., 2017) or other agricultural stressors (Salis et al., 2017), and gradients of eutrophication and urban contamination in freshwater systems (Apothéloz-Perret-Gentil et al., 2017; Martínez-Santos et al. 2018; Simonin et al., 2019; Tapolczai et al., 2019a, 2019b). In marine environments, the utility of this strategy has been demonstrated after an oil spill (Bik et al., 2012), in the vicinity of offshore drilling platforms (Lanzén et al., 2016; Laroche et al., 2016, 2018a) and aquaculture sites (Pawlowski et al. 2014, Pochon et al., 2015; Dowle et al., 2015; Keeley et al., 2018; Stoeck et al. 2018a, 2018b) as well as along eutrophication and urban or industrial contamination gradients in estuaries (Chariton et al., 2010, 2015; Angly et al., 2015; Lallias et al., 2015; Obi et al., 2016). Interestingly, most of the studies sampling marine sediments highlighted that meiofaunal invertebrates, such as nematodes, gastrotrichs and platyhelminths (Chariton et al., 2010; Bik et al., 2012; Lanzén et al., 2016), large groups of protists such as diatoms, oomycetes and ciliates (Lanzén et al., 2016; Stoeck et al., 2018a) or foraminifera (Pawlowski et al., 2014; Laroche et al., 2016; Frontalini et al., 2018) but also fungi

(Bik et al., 2012) and bacteria (Angly et al., 2015; Dowle et al., 2015; Martínez-Santos et al. 2018; Obi et al., 2016; Aylagas et al., 2017; Stoeck et al., 2018b; Keeley et al. 2018) have great potential as bioindicators of anthropogenic impacts and can readily be captured by EG studies.

Unfortunately, most proof-of-concept studies employing the *de novo* strategy have not yet validated their results by performing ecological assessments based on newly identified bioindicators as a reference in a new environmental context. For this information to be useful on new samples, the data obtained from known disturbance gradients (i.e. reference or training dataset) must be operational in different spatiotemporal contexts. To this end, two main approaches have been proposed and tested, namely indicator value (e.g. the IndVal approach, Dufrêne and Legendre 1997) and supervised machine learning (SML, Crisci et al., 2012; Libbrecht & Noble, 2015).

The indicator value approach ascribes autecological values (or discrete “eco-groups”) to OTUs or ASVs based on their occurrence in samples of known disturbance level, in a similar manner as for the establishment of morphology-based bioindicators. Hence, the autecological values of these *de novo* bioindicators are directly calibrated on the HTS data, which alleviates the qualitative and quantitative biases encountered with the taxonomy-based EG strategy. This has proven successful for both freshwater benthic diatoms (Apothéloz-Perret-Gentil et al., 2017; Tapolczai et al., 2019a, 2019b) and for bacterial and eukaryotic communities in streams and estuarine systems (Chariton et al., 2015; Li et al., 2018). An analogous approach is the use of polynomial quantile regression splines (Andersson, 2008). This has shown great promise for the prediction of impacts from organic enrichment in aquaculture sites using eukaryotic and prokaryotic metabarcoding data in parallel (Keeley et al., 2018). For diatoms, the accuracy of the assessment can be largely improved, arguably because the indicator value approach makes use of a larger number of OTUs or ASVs, compared to an approach relying solely on their taxonomic assignments (Apothéloz-Perret-Gentil et al., 2017; Tapolczai et al. 2019a, 2019b).

Supervised machine learning (SML) also requires training datasets, i.e. reference disturbance levels (labels) associated with the community profiles of the samples (features). These algorithms are best at classification problems involving multidimensional and noisy datasets (Libbrecht & Noble, 2015), which are common attributes of HTS data. The task is to automatically disentangle the feature signal (OTU or ASV profiles) and their co-occurrence that convey an ecological signal from background noise. This extracted knowledge is self-contained

in a trained model that can be used to make predictions of disturbance level on new samples, based on their compositional profiles (Cordier et al., 2019a). Supervised machine learning also alleviates the qualitative and quantitative biases that hamper the taxonomy-based strategy in a more straightforward manner, because the model is trained directly on HTS data. The applicability of SML has been demonstrated in marine environments, for the detection of various pollutants (Smith et al., 2015) and for the prediction of aquaculture impacts on benthic biodiversity (Cordier et al., 2017; 2018). The SML-based inference of BI values has also been shown to outperform the taxonomy-based strategy, relying on the detection of established macroinvertebrates bioindicators DNA (Cordier et al., 2018), and may be more powerful that the IndVal approach (Frühe et al., 2020). Supervised machine learning applications have also succeeded in predicting the origin of container ship ballast waters (Gerhard & Gunsch, 2019).

The *de novo* strategy provides numerous advantages over the taxonomy-based one. First, it can reduce or bypass the dependence on reference sequence databases for taxonomic assignments of HTS reads to known bioindicators. Instead, new ecological knowledge is hypothesised *de novo* during the calibration of OTUs or ASVs autecological values (IndVal) or during the supervised training of a model (SML). Second, it can leverage powerful but previously inaccessible groups of bioindicators among prokaryotes, protists, meiofauna and mesozooplankton, that are widespread and may react both faster and stronger to environmental disturbances (Creer et al., 2010; Payne, 2013; Bouchez et al., 2016; Pawlowski et al. 2016). Finally, when applied for the inference of BIs that are currently employed in routine monitoring programs, a *de novo* strategy is directly compatible with current regulations, because the assessment categories remain the same and the BI values are simply inferred indirectly. Hence, this strategy assures a full backward and forward compatibility with current monitoring programs, facilitating continuity of important time series datasets (Bálint et al., 2018).

“Structural community metrics” strategy: blending theoretical ecology into routine ecosystem monitoring.

This strategy relies on metrics extracted from the community structure or from inferred ecological networks – where taxa are interchangeable – in order to assess the impact of disturbance and its ramifications on ecosystem functioning (Figure 1C). This represents a clear

paradigm-shift for ecosystem monitoring programs, because the evaluation of bioindicators, based on the compositional variation of communities, is not the main aim of the strategy. Instead, its focus is to discover and understand the ecological processes shaping biological communities and their response to disturbances, which is indeed one of the core questions of ecological research. It has long driven the exploration of the links between generic, taxonomy and composition-independent biodiversity metrics or species functional traits distribution and ecosystems functioning and resilience, to reach a more general theoretical framework (Cardinale et al., 2000; McCann, 2000; Hooper et al., 2005; Tilman et al., 2006; Ives & Carpenter, 2007; Mouillot et al., 2013; Loreau & de Mazancourt, 2013).

Structural community metrics can be computed from compositional data generated by EG studies, including alpha diversity (e.g. OTU or ASV richness, Shannon diversity or Pielou evenness; reviewed in Daly et al., 2018), along with its phylogeny-aware derivatives (reviewed in Tucker et al., 2017; Washburne et al., 2018). Under anthropogenic impact, alpha diversity in marine sediment has been found to decrease for foraminifera (Pawlowski et al., 2014; 2016; Laroche et al. 2018b), ciliates (Stoeck et al., 2018a) and bacterial communities (Stoeck et al., 2018b). Conversely, disturbances in marine sediments can also trigger increases in bacterial diversity and metabolic activity (Galand et al., 2016; Pérez-Valera et al., 2017). This suggests that the variation of alpha diversity alone is insufficient as a widely applicable indicator of disturbance. Phylogeny-aware metrics attempt to account for the evolutionary relationships among taxa composing communities, to provide insights into community assembly processes and by extension their predictable responses to environmental variations (Webb et al., 2002; Cavender-Bares et al., 2009, but see Mayfield & Levine, 2010; Gerhold et al., 2015). This relationship between phylogenetic diversity and ecosystem functioning has received a lot of attention by plant ecologists (Flynn et al., 2011). However, only few studies have employed EG data to this end, targeting mostly microbial groups, which, as for simple alpha-diversity metrics, has resulted in contrasting conclusions (Galand et al., 2015; Pérez-Valera et al., 2017, Liu et al., 2017; but see Venail & Vives, 2013; Keck & Kahlert, 2019 for studies employing sequencing data but not strictly EG).

Metrics based upon alpha diversity may be misleading (Santini et al., 2017) because their variation is often non-linear, strongly scale-dependent (Chase et al., 2019) and valuable only in comparing contexts sampled using the same methodology (Shade, 2017). It also

implicitly conveys the idea that ‘higher diversity is better’ which is not necessarily true (Shade, 2017). The inference of ecological functioning based on phylogeny-aware metrics relies on the niche conservatism concept, which postulates that closely related taxa share similar functional traits (Webb et al., 2002; Cavender-Bares et al., 2009; Srivastava et al., 2012). Under this assumption, increased phylogenetic diversity may support functionally diverse or multifunctional ecosystems (Hector & Baghi, 2007 but see Manning et al., 2018). By extension, higher phylogenetic diversity may also support ecosystem resilience, provided that the species fulfilling similar functions have differing responses to disturbances (Cadotte et al., 2012; Oliver et al., 2015). However, because not all functional traits necessarily have a phylogenetic signal (Srivastava et al., 2012), including for microbes (Martiny et al., 2013), inferring ecosystem functioning and the level of anthropogenic impact based on phylogeny-aware metrics alone may prove to be misguided. Likewise, conservation strategies based on these metrics may also be suboptimal (Mazel et al., 2018).

Another set of structural community metrics can be computed from the topology of inferred ecological or co-occurrence networks, representing potential biotic interactions (reviewed in Faust & Raes, 2012; Vacher et al., 2016; Layeghifard et al., 2017). Based on empirical evidence of the variation in network structure under environmental disturbance (Tylianakis et al., 2007; Zhou et al., 2011; Karimi et al., 2016; Ma et al., 2018), their properties have been suggested as potential indicators of ecosystem functioning and integrity (Gray et al., 2014; Karimi et al., 2017; Bohan et al., 2011, 2017; Lau et al., 2017; Tylianakis et al., 2017; Pellissier et al., 2018; Delmas et al., 2019). In recent years, a growing interest in these approaches has led to a series of studies employing EG to infer ecological networks from microbial community data (Zhou et al., 2011; Lupatini et al., 2014; Zappelini et al., 2015; Pérez-Valera et al., 2017; Pauvert et al., 2019) or from macroinvertebrates (Compson et al., 2019), in order to explore the links between network properties such as connectance, centrality or nestedness, and ecosystem functioning. For instance, it has been shown that bacterial communities in anthropized soil may have fewer potentially interacting taxa, than in natural soil (Lupatini et al., 2014). Likewise, in aquatic ecosystems, anthropogenic impacts are reflected in co-occurrence networks by a lower connectivity (Lawes et al., 2017; Laroche et al., 2018b; Li et al., 2018) and a lower ratio of positive interactions (Laroche et al., 2018b).

While promising, exploring the links between the properties of ecological networks inferred from EG data and ecosystem functioning is still in its infancy (Faust et al., 2012; 2015; Lima-Mendez et al., 2015; Lawes et al., 2017; Laroche et al., 2018b; Li et al., 2018; Pauvert et al., 2019). Multiple methodological issues limit the inference of robust networks from EG data based on co-occurrences in space or time. For example, read counts are strictly compositional, representing relative abundance of the marker itself, rather than presence or absolute abundances (but see Friedman & Alm, 2012; Kurtz et al., 2015). Further, it is challenging to control for covariates and confounding environmental parameters (but see Tammadoni-Nezhad et al., 2013; Tackmann et al., 2018; Cougoul et al., 2018; Chiquet et al., 2018; Momal et al., 2019), replicability of inference (Pauvert et al., 2019) and the relative merits of statistical and logical inference (Vacher et al., 2016). Robust networks also require considerably more replicates than are typically collected in EG studies, which increase both time and costs. Nevertheless, as more benchmark datasets containing both EG data and independently confirmed interactions between taxa become available to complement simulated datasets (see Lima-Mendez et al., 2015), making robust network inference to explore the applicability of their metrics for ecosystem monitoring will likely come within reach in the years to come.

"Functional community metrics" strategy: employing functional environmental genomics for routine monitoring.

Another avenue of implementation of EG for ecosystem monitoring is the use of shotgun metagenomics and metatranscriptomics, depicting the metabolic capabilities of the community, and the expressed genes at the moment of sampling, respectively (Figure 1D). However, ecologists have yet to disentangle the relative importance and relationship of taxonomic diversity and functional traits for ecosystems functioning (Flynn et al., 2011; Gagic et al., 2015). This is particularly true in microbial ecology with the "who's there" *versus* "what they are doing" paradigms that often relate to the employed molecular methodologies, i.e metabarcoding versus metagenomics and metatranscriptomics (Xu et al., 2014). Some metagenomic contigs and functional transcripts were indeed found to represent efficient bioindicators of anthropogenic disturbances (Table S1), in terrestrial (de Menezes et al., 2012), groundwater (He et al., 2018), freshwater (Thompson et al., 2016; Cheaib et al., 2018; Falk et al., 2019) and marine environments (Kisand et al., 2012; Galand et al., 2016; Birrer et al., 2019), opening up potential

avenues for future routine ecosystem monitoring applications. Functional and taxonomic profiles may respond differently under anthropogenic disturbance (Cheaib et al., 2018), as well as under natural environmental variation (Barberàn et al., 2012; Louca et al., 2016a; 2016b; Louca et al., 2018). This taxon-function decoupling paves the way towards a molecular trait-based ecology (Raes et al., 2011; Lajoie & Kembel 2019).

In an ecosystem monitoring context, functional profiles present two important features that anticipate these proxies to be more accurate than taxonomic profiles for the detection of a given environmental disturbance. First, because prokaryotes functional redundancy may be widespread (Louca et al., 2018; Pearman et al., 2019; but see Galand et al., 2018 and see Ramond et al. 2019 for protists), any given anthropogenic disturbance might trigger a similar response across multiple taxonomic groups. Under this assumption, ecosystem monitoring based on functional profiles may be less sensitive to biogeographical effects, random demographic drift, and species dispersal limitation than a monitoring strategy based on taxonomic profiles. This functional redundancy would also allow the establishment of a direct and mechanistic link between a measured functional response to a given anthropogenic disturbance. Second, because functional shifts are likely to occur prior to compositional ones, as a response of the taxa present to the disturbance, the variation of functional profiles may constitute useful early warnings for a timelier ecosystem management, especially the ones detected by means of metatranscriptomics. However, RNA molecules are reportedly less stable than genomic DNA, which would add challenging practical constraints that could preclude their implementation in routine ecosystem monitoring programs (but see Fordyce et al., 2013; Pochon et al., 2017; Cristescu, 2019; von Ammon et al. 2019). As a possible cost-effective “shortcut”, bacterial 16S rRNA profiles can be used to predict functional community profiles, based on evolutionary models (Langille et al., 2013; Aßhauer et al., 2015). Thus, 16S data could be also explored for searching potential functional bioindicators by this approach (Mukherjee et al., 2017; Laroche et al., 2018; Cordier 2020).

A roadmap for the implementation of environmental genomics for ecosystem monitoring

The emergence of standards for EG methodologies to be applied for monitoring programs.

The time lag between technological breakthroughs, the uptake by scientists and the implementation of research results into real management applications can be notoriously long. Even for clinical applications where the contributions of genomics have long been anticipated (Dulbecco, 1986; Manolio et al., 2013) and for which economic perspectives are obvious, its implementation for routine healthcare applications is considered to have started five years ago (Stark et al., 2019). This is three times faster than the average 17 years for any healthcare research (Morris et al., 2011). The emergence of consensual standards for methodological protocols and data formats for interoperable exchanges, represent the most challenging issue for the routine adoption (Stark et al., 2019).

The field of EG for ecosystems monitoring is experiencing similar issues and has yet to overcome some of the barriers to the necessary paradigm-shift in monitoring programs (Hering et al., 2018). Some of the noteworthy steps towards this goal were achieved with the widespread adoption of the MIGS, MIMARKS and MIxS standards in genomics, specifying the minimum information that should accompany any genome, marker gene sequences or any sequence (Field et al., 2008; Yilmaz et al., 2011). Now the most challenging part resides in the adoption of standardized methodologies to produce, store and analyze EG data for a given environmental setting. Given the variety of biological models and environmental matrices, reaching a consensus in the scientific community and formalizing standards appears very challenging, especially for metabarcoding (Pollock et al., 2018; Knight et al., 2018; Wilcox et al., 2018; Zinger et al., 2019) and its application to ecosystem monitoring (Cristescu & Hebert, 2018; Hering et al., 2018). Yet, these hurdles are not specific to genomics methodologies, but also exist for the morphology-based ones (Birk et al., 2012). Building robust, shared methodological standards is of course necessary and important efforts are deployed to reach this aim (Leese et al., 2018; Hering et al., 2018; Working Group CEN/TC230/WG28), for the sampling of eDNA (Dickie et al., 2018; Wilcox et al., 2018; CEN 2018a), the molecular protocols (Goldberg et al., 2016; Blackman et al., 2019) as well as for bioinformatics (Roy et al., 2018;

Knight et al., 2018), data interoperability (McDonald et al., 2012; Callahan et al., 2017) and reference databases (CEN, 2018b).

Matching the right implementation strategy to the right monitoring program.

Several monitoring programs may benefit quickly and reliably from an EG implementation, while others may require further optimization of molecular protocols or adjustments of their assessment criteria (Table 1). For instance, monitoring programs relying primarily on taxonomic inventories are still hindered by the lack of congruence between the recovered species list and their relative abundances, even though the biological and technical biases might be partially alleviated in the future. Furthermore, despite the sustained effort, reference sequence databases for barcoding remain skewed toward some groups and geographical locations (Weigand et al., 2019; McGee et al., 2019), limiting congruence between EG and morpho-taxonomic inventories. Hence, the taxonomy-based implementation strategy for these monitoring programs will require improvements of molecular protocols and reference databases, to generate EG data that better fit the current standards, or an adaptation of the currently implemented assessment criteria to fit the specificities of EG data (Hering et al., 2018).

Monitoring programs relying on the screening of established bioindicators for the computation of BI values are proposed as being compatible with an implementation of EG (Hering et al., 2018; Pawlowski et al., 2018). Indeed, this compatibility is greatly facilitated by the fact that the assessment criteria, i.e. BIs, are not meant to strictly rely on taxonomic inventories but rather on the autecology of bioindicators. Hence, for the taxonomy-based strategy, the BI formulations can compensate the impact of taxonomic mismatches between morphology and EG and databases incompleteness to some extent, because multiple taxa are ascribed identical autecological values, conveying similar ecological signal (Keck et al., 2018). The applicability of this approach has been demonstrated in freshwater (Elbrecht et al., 2017; Vasselon et al., 2017b; Kelly et al., 2018; Mortagua et al., 2019; Rivera et al., 2020) and in marine environments (Lejzerowicz et al., 2015; Aylagas et al., 2016). However, those studies have also shown that a large amount of sequences are not taxonomically assigned and currently omitted for ecological assessment, opening the door to new approaches that could extract ecological information from those unlabeled sequences.

552 The *de novo* strategy uses the occurrence of previously scrutinized sequences in
553 samples of known BI values or other impact measures to ascribe autecological values to
554 sequences directly, or generate a predictive model (Apothéloz-Perret-Gentil et al., 2017; Cordier
555 et al., 2017; Tapolczai et al. 2019). Hence, these approaches are less sensitive to the biological
556 and technical issues mentioned above, because the ecological signal (autecology) is calibrated
557 directly on the specificities of EG data. From an implementation perspective, this *de novo*
558 strategy thus may represent the most direct path towards implementation of EG into monitoring
559 programs relying on BIs (Figure 2). Though somewhat unintuitive, this is because inferred BI
560 values with a *de novo* strategy convey the same ecological meaning as they do with current
561 methodologies, which is not the case when BIs values are inferred from bioindicators
562 composition profiles depicted by EG data, as their autecological values were calibrated only on
563 morphology-based data. Thus, the *de novo* strategy assures a better continuity with previous
564 BIs data and time series and expand the range of possible bioindicators to virtually any taxa or
565 sequence.
566 Structural and functional community metrics represent alternative implementation
567 strategies that may ultimately lead to a more generic, broadly applicable ecological monitoring
568 framework (Bohan et al., 2017; Karimi et al., 2017; Tylianakis et al., 2017; Quince et al., 2017;
569 Singer et al., 2017; Pellissier et al., 2018; Escalas et al., 2019). These strategies hold the
570 potential to provide a more mechanistic and functional understanding of the response of
571 biological communities to ecosystem variation. Such knowledge could hence be included in
572 predictive models to forecast shifts in biodiversity structure and possibly their consequences on
573 their associated ecosystem services under different disturbance scenarios. However, an
574 operational ecosystem monitoring framework remains to be built upon this theoretical ecological
575 work (Figure 2), that has only partially been experimentally validated (but see Laroche et al.,
576 2018; Ma et al., 2019). In addition, the extraction of structural or functional community metrics
577 remain active fields of ecological research, and the emergence of a molecular trait-based
578 ecology using metagenomics and metatranscriptomics profiles is in its infancy (Lajoie et al.,
579 2019). Hence, it is premature to discuss their operational implementation and regulatory
580 establishment, but their ecological benefit should be anticipated. Nevertheless, the collected
581 labelled datasets including samples for the production of EG data in the course of future
582 ecosystem monitoring campaigns will certainly contribute to move these possibilities forward.

Collecting reference data and eDNA/eRNA samples in parallel.

If EG-based methods are to complement or replace current morphology-based ones, the prerequisite is to establish whether they can provide similar ecological diagnostics, to ensure a smooth implementation and compatibility with existing time series (Leese et al., 2016; Bálint et al., 2018). This inevitably implies extensive parallel sampling of currently implemented and EG methodologies for some time, to build reference datasets on which the applicability can be assessed and the calibration with previous methodology performed (Leese et al., 2016; Keeley et al. 2018). To be reliable, such reference datasets have to cover a broad range of possible environmental conditions for a given ecosystem across multiple spatiotemporal scales, ideally in a balanced manner, to account for biotic interactions, random demographic drift and dispersal limitations that may interact with the anthropogenic pressures in the assembly of communities.

The collection of reference data raises concerns regarding the substantial financial investment necessary for monitoring programs adopting one or a combination of EG strategies, *versus* the “risk” of technological novelty and/or paradigm-shift. However, the collected reference datasets would still be extremely valuable in such case, because the extracted DNA/RNA alongside the accompanying reference metadata can be safely stored and re-analysed later on, assuring a forward compatibility to the limit of availability of stored DNA/RNA material (Hering et al., 2018; Jarman et al., 2018). Indeed, molecular costs are usually far less prohibitive than those related to field sampling and metadata collection. Hence, such fully labelled datasets will constitute the ideal benchmarks against which to assess the validity of any new implementation strategy based on novel technology or new paradigm.

Conclusion and further research needs

The potential for EG-based methods for ecosystems monitoring is enormous and can presently fulfil most of the requirements of current monitoring programs. Moving towards a routine use of EG is certainly a paradigm-shift, but this technological breakthrough will

overcome the limitations of current morpho-taxonomy methodologies and enable the required up-scaling to meet monitoring needs in a changing world. Without doubts, EG-based methods will pave the way for a more cost-effective, faster, reproducible and semi-automatable ecosystem monitoring framework. Regardless of the implementation strategy envisioned, the following key technological, scientific and societal improvements will be beneficial for a smoother transition:

- A collaborative and transdisciplinary design of monitoring campaigns, involving both experts, stakeholders and regulators would allow monitoring programs to more easily bridge the science-policy gap.
- A collection of reference morphological and molecular data in parallel, at least in a subset of reference points or during a transition period, will assure backward and forward compatibility of time series datasets, regardless of the envisioned implementation strategy to be decided in future monitoring campaigns.
- The efforts to complete reference sequence databases need to be sustained, by adding more representatives of the known biodiversity, with a wider geographical coverage.
- A reference database framework for *de novo* strategies needs to be established. A key requirement is the ability to reliably compare OTUs or ASVs identified in monitoring programs to formally establish knowledge about their sensitivity to disturbance.
- The taxonomic resolution level (haplotype, species, genus, family, order, class) at which HTS reads are most informative as genetic bioindicators for a given situation remains to be identified.
- For the identification of novel genetic bioindicators in complex communities, it will be important to distinguish the effect of natural (seasonal) variation from disturbance-induced community changes with rigorous experimental designs.
- Basic and replicable research is highly needed to develop a structural and functional community metrics-based implementation strategy. Such effort will likely contribute to the establishment of a more broadly applicable monitoring framework and less constrained by the database and geographical coverage limitations.

Box 1: Glossary of terms used in this paper

- Implementation strategy: Refers to the way environmental genomics data is produced and analysed in an ecosystem monitoring context. It includes the choice of all the molecular biology steps, i.e. targeted molecules (DNA versus RNA), metabarcoding (amplicon sequencing) versus metagenomics or metatranscriptomics (shotgun sequencing), and the computational biology steps (analytical approach), i.e. focusing on the taxonomically assigned sequences or considering all the sequences, the use of compositional turnovers (beta-diversity), structural metrics (alpha or phylogenetic diversity and ecological network properties) or functional metrics (functional genes or transcripts diversity).
- Environmental genomics: Suite of molecular tools to sample, process and analyse nucleic acids from an environmental sample (soil, water, sediment, feces)
- Environmental DNA/RNA: Nucleic acids present in an environmental sample. It encompasses the DNA/RNA within living multi or unicellular organisms, dead or decaying as well as extracellular material.
- Metabarcoding: A molecular workflow to simultaneously study the diversity of PCR-selected organisms from environmental samples using high-throughput sequencing. This is equivalent to amplicon sequencing of a taxonomic marker.
- Metagenomics: Shotgun sequencing of the genomic DNA isolated from an environmental sample. There is no PCR selection of particular taxonomic group and include coding as well as non-coding genomic material.
- Metatranscriptomics: Shotgun sequencing of retro-transcribed RNA isolated from an environmental sample. As for metagenomics, there is no PCR selection but includes only transcribed RNA (mRNA, rRNA), supposedly functional.
- Bioindicator: A taxon, marker sequence, gene or transcript that is used as an indicator of the ecological status of an environment.
- Autecological value: Ecological knowledge about the distribution and abundance of particular species obtained by studying interactions of individual organisms with their environments.
- Biotic Indices: Continuous or discrete variables that measure the level of disturbance of an environment based on the composition and relative abundance of bioindicator taxa (or OTUs/ASVs). Around half of the existing monitoring programs rely on biotic indices (BIs). The BIs usually includes several ordered discrete classes, usually from ‘poor’ to ‘high’ ecological status.

- Ecological network: Representation of statistically inferred biotic interactions through spatial or temporal co-occurrence or co-exclusion. Taxa (nodes) are connected by pairwise links (edges). Network ecology aims to understand how these network properties are linked to the functioning of ecosystems.

Figures and tables

Figure 1: Overview of the current methodology for the monitoring of ecosystems, that relies mostly on the morphological identification of biodiversity and / or bioindicators of anthropogenic impacts. Ecological diagnostics are performed based on reference biodiversity or on reference biotic indices for a given ecosystem. The development of environmental genomics methodologies has led to the proposition of multiple implementation strategies that can intervene at different levels of the monitoring workflow, to produce an ecological diagnostic. Green colors and smileys within boxes indicate reference or “high” ecological status while red colors and smileys represent non-reference biodiversity or “poor” ecological status (i.e. impacted environments). The colors on tags besides organisms or sequences indicate their bio-indication value (red: indicator of impact, yellow: indicator of intermediate status, green: indicator of good status). In this review paper, these strategies have been grouped in four broad categories: (A) Taxonomy-based analyses focused on identification of known bio-indicators or described taxa; (B) *De novo* bioindicator analyses; (C) Structural community metrics including inferred ecological networks; and (D) Functional community metrics (metagenomics or metatranscriptomics).

Figure 2: Strengths and limitations of the currently envisioned implementation strategies of environmental genomics for the monitoring of ecosystems, and their ability to fulfill the criteria of existing monitoring programs.

Table 1: Comparison of the four implementation strategies in terms of compatibility with current standards, backward and forward compatibility, performance, biodiversity coverage, generalization potential and ease of standardization

Table S1: List of studies employing environmental genomics for ecosystem monitoring sorted by strategy, ecosystem, targeted taxonomic group and objective.

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